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(54) **Echinocandin binding domain of 1,3-Beta-glucan synthase**

(57) The invention relates to a substantially purified ECB binding domain of 1,3- β -glucan synthase, comprising an at least 46 amino acid peptide fragment or fusion

protein of glucan synthase that binds echinocandins, useful in a method for identifying new antifungal compounds. Also disclosed are nucleic acid molecules that encode said peptide.

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Description

[0001] This invention claims the benefit of U.S. Provisional Application No. 60/068,658, filed December 23, 1997.

[0002] This invention relates to recombinant DNA technology. In particular the invention pertains to a fungal glucan synthase, and to a sub-region thereof that mediates echinocandin binding and antifungal activity. Also contemplated is the use of said echinocandin binding region in screens for compounds that bind glucan synthase.

[0003] The incidence of life-threatening fungal infections is increasing at an alarming rate. About 90% of nosocomial fungal infections are caused by species of *Candida*, with the remaining 10% being attributable to *Aspergillus*, *Cryptococcus*, and *Pneumocystis*. While effective antifungal compounds have been developed for *Candida*, there is growing concern over escalating resistance in other pathogenic fungi. Since *anti-Candida* compounds rarely are clinically effective against other fungi, new compounds are needed for effective antifungal therapy.

[0004] The present invention provides an echinocandin binding domain of a fungal 1,3,β-glucan synthase (hereinafter "glucan synthase") that is useful in identifying compounds that bind and inhibit glucan synthase activity. The compositions of this invention enable identification of new and better antifungal compounds.

[0005] In one embodiment the present invention relates to a nucleic acid molecule that encodes an echinocandin binding domain of fungal glucan synthase.

[0006] In another embodiment the present invention relates to a peptide that comprises an echinocandin binding site of fungal glucan synthase.

[0007] In another embodiment, the present invention relates to a method for identifying compounds that bind an echinocandin binding domain of fungal glucan synthase.

[0008] "ECB binding domain" or "ECB binding site" or "ECB binding fragment" refers to a subregion of the yeast glucan synthase molecule (i.e. product of *FKS1* gene in *S. cerevisiae*), wherein said subregion retains, either alone or in combination with another protein, for example, as a fusion protein, the capacity to bind echinocandins such as ECB. For example, in one embodiment the present invention relates to a subregion of SEQ ID NO:2 comprising amino acid residues 583 to 672. ECB binding fragments may be verified by any suitable test for binding to ECB or other echinocandin, or papulocandin, or related compounds.

[0009] The term "fusion protein" denotes a hybrid protein molecule not found in nature comprising a translational fusion or enzymatic fusion in which two or more different proteins or fragments thereof are covalently linked on a single polypeptide chain.

[0010] The term "plasmid" refers to an extrachromosomal genetic element. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accordance with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

[0011] "Recombinant DNA cloning vector" as used herein refers to any autonomously replicating agent, including, but not limited to, plasmids and phages, comprising a DNA molecule to which one or more additional DNA segments can or have been added.

[0012] The term "recombinant DNA expression vector" as used herein refers to any recombinant DNA cloning vector, for example a plasmid or phage, in which a promoter and other regulatory elements are present to enable transcription of the inserted DNA.

[0013] The term "vector" as used herein refers to a nucleic acid compound used for introducing exogenous DNA into host cells. A vector comprises a nucleotide sequence which may encode one or more protein molecules. Plasmids, cosmids, viruses, and bacteriophages, in the natural state or which have undergone recombinant engineering, are examples of commonly used vectors.

[0014] The terms "complementary" or "complementarity" as used herein refers to the capacity of purine and pyrimidine nucleotides to associate through hydrogen bonding in double stranded nucleic acid molecules. The following base pairs are complementary: guanine and cytosine; adenine and thymine; and adenine and uracil.

[0015] "Isolated nucleic acid compound" refers to any RNA or DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location.

[0016] A "primer" is a nucleic acid fragment which functions as an initiating substrate for enzymatic or synthetic elongation of, for example, a nucleic acid molecule.

[0017] The term "promoter" refers to a DNA sequence which directs transcription of DNA to RNA.

[0018] A "probe" as used herein is a labeled nucleic acid compound which hybridizes with another nucleic acid compound.

[0019] The term "hybridization" as used herein refers to a process in which a single-stranded nucleic acid molecule joins with a complementary strand through nucleotide base pairing. "Selective hybridization" refers to hybridization under conditions of high stringency. The degree of hybridization depends upon, for example, the degree of complementarity, the stringency of hybridization, and the length of hybridizing strands.

[0020] The term "stringency" refers to hybridization conditions. High stringency conditions disfavor non-homologous

basepairing. Low stringency conditions have the opposite effect. Stringency may be altered, for example, by temperature and salt concentration.

[0021] "Low stringency" conditions comprise, for example, a temperature of about 37° C or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or, alternatively, a temperature of about 50° C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

[0022] "High stringency" conditions comprise, for example, a temperature of about 42° C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65° C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. *et al.* Current Protocols in Molecular Biology, Vol. 1, 1989; Green Inc. New York, at 2.10.3).

[0023] "SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

[0024] "SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM Na₂HPO₄, 0.9 mM NaH₂PO₄ and 1 mM EDTA, pH 7.4.

[0025] "Substantially pure" used in reference to a peptide or protein means that said peptide or protein is separated from a large fraction of all other cellular and non-cellular molecules, including other protein molecules. A substantially pure preparation would be about at least 85% pure; preferably about at least 95% pure. For example, a "substantially pure" protein as described herein could be prepared by the IMAC protein purification method, or any other suitable method.

[0026] Cell walls are essential to the viability of fungi, but have no existence in mammalian cells. This makes synthesis of the fungal cell wall a useful target for antifungal compounds. Two polysaccharide polymers, chitin and 1,3-β-glucan, are essential components of fungal cell walls. Therefore, antibiotics that interfere with the synthesis of these polymers are useful in mycosis therapy. Polysaccharides have been estimated to account for as much as 80% to 90% of the *Saccharomyces cerevisiae* cell wall. The major cell wall polymers are glucan and mannan, and small amounts of chitin.

[0027] In *S. cerevisiae*, cell wall synthesis is thought to involve at least a subunit of glucan synthase, which is encoded by the *FKS1* gene (Douglas *et al.* *Proc. Nat. Acad. Sci.* 91, 12907-911, 1994). *FKS1* encodes a 215 kD integral membrane protein of 1876 amino acid residues that is the likely target of ECB and other echinocandins (*Id.*) For example; resistance to ECB and other echinocandins maps to the *FKS1* locus. More specifically, a domain of *FKS1*, which resides at amino acid residues 583 to 672 defines a cytoplasmic loop thought to be necessary and sufficient to comprise an echinocandin binding domain.

Gene Isolation Procedures

[0028] Those skilled in the art will recognize that the nucleic acids of this invention may be obtained by a plurality of applicable genetic and recombinant DNA techniques including, for example, polymerase chain reaction (PCR) amplification, or *de novo* DNA synthesis. (See e.g., J. Sambrook *et al.* *Molecular Cloning*, 2d Ed. Chap. 14 (1989)).

[0029] Skilled artisans will recognize that a nucleic acid encoding the ECB binding domain could be isolated by PCR amplification of any suitable genomic DNA or cDNA using oligonucleotide primers targeted to the appropriate region of *FKS1* (*viz.* encoding amino acid residues 587 to 672 of SEQ ID NO:2). The preferred template source for PCR amplification is *S. cerevisiae* genomic DNA. Methods for PCR amplification are widely known in the art. See e.g. PCR Protocols: A Guide to Method and Application, Ed. M. Innis *et al.*, Academic Press (1990). The amplification reaction comprises genomic DNA, suitable enzymes, primers, and buffers, and is conveniently carried out in a DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, CT). A positive result is determined by detecting an appropriately-sized DNA fragment following agarose gel electrophoresis.

Protein Production Methods

[0030] The present invention also relates to a substantially purified peptide, or fusion protein, comprising a sub-region of glucan synthase that functions as an echinocandin binding site.

[0031] Skilled artisans will recognize that the proteins and peptides of the present invention can be synthesized by any number of different methods including solid phase chemical synthesis or recombinant methods. Both methods are described in U.S. Patent 4,617,149, incorporated herein by reference.

[0032] The principles of solid phase chemical synthesis are well known in the art and may be found in general texts in the area. See, e.g., H. Dugas and C. Penney, Bioorganic Chemistry (1981) Springer-Verlag, New York, 54-92. For example, peptides may be synthesized by solid-phase methodology utilizing an Applied Biosystems 430A peptide synthesizer (Applied Biosystems, Foster City, CA) and synthesis cycles supplied by Applied Biosystems. Protected amino acids, such as t-butoxycarbonyl-protected amino acids, and other reagents are commercially available from many chemical supply houses.

[0033] The peptide of the present invention can also be produced by recombinant DNA methods using a cloned nucleic acid. Recombinant methods are preferred if a high yield of the peptide is desired. Expression of a cloned nucleic acid can be carried out in a variety of suitable hosts, well known to those skilled artisan. For example, the cloned DNA is introduced into a host cell by any suitable means, well known to those skilled in the art. While chromosomal integration of the cloned nucleic acid is within the scope of the present invention, it is preferred that it comprise part of a suitable extra-chromosomally maintained expression vector.

[0034] The basic steps in the recombinant production of the peptides of this invention are:

- a) constructing a natural, synthetic or semisynthetic DNA encoding said protein, peptide, or fusion protein;
- b) integrating said DNA into an expression vector in a manner suitable for expressing the protein, either alone or as a fusion protein;
- c) transforming or otherwise introducing said vector into an appropriate eucaryotic or prokaryotic host cell, forming a recombinant host cell,
- d) culturing said recombinant host cell in a manner to express the protein; and
- e) recovering and substantially purifying the protein by any suitable means.

Expressing a Recombinant ECB Binding Domain in Prokaryotic and Eucaryotic Host Cells

[0035] In general, procaryotes are used for cloning DNA sequences and for constructing the vectors of the present invention. Procaryotes may also be used in the production of the ECB binding peptide. For example, the *Escherichia coli* K12 strain 294 (ATCC No. 31446) is particularly useful for the prokaryotic expression of foreign proteins. Other strains of *E. coli*, bacilli such as *Bacillus subtilis*, enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcescans*, various *Pseudomonas* species and other bacteria, such as *Streptomyces*, may also be employed as host cells in the cloning and expression of the recombinant proteins of this invention.

[0036] Promoter sequences suitable for driving the expression of genes in procaryotes include β -lactamase [e.g. vector pGX2907, ATCC 39344, contains a replicon and β -lactamase gene], lactose systems [Chang et al., *Nature* (London), 275:615 (1978); Goeddel et al., *Nature* (London), 281:544 (1979)], alkaline phosphatase, and the tryptophan (trp) promoter system [vector pATH1 (ATCC 37695) which is designed to facilitate expression of an open reading frame as a trpE fusion protein under the control of the trp promoter]. Hybrid promoters such as the tac promoter (isolatable from plasmid pDR540, ATCC-37282) are also suitable. Still other bacterial promoters, whose nucleotide sequences are generally known, enable one of skill in the art to ligate such promoter sequences to DNA encoding the proteins of the instant invention using linkers or adapters to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno sequence operably-linked to the DNA encoding the desired polypeptides. These examples are illustrative rather than limiting.

[0037] The peptides of this invention may be synthesized *de novo*, or they may be produced as a fusion protein comprising the peptide of interest (viz. ECB binding fragment) as a translational fusion with another protein or peptide that may be removable by enzymatic or chemical cleavage. It is often observed that expression as a fusion protein prolongs the lifespan, increases the yield of a desired peptide, and provides a convenient means of purifying the protein. A variety of peptidases (e.g. enterokinase and thrombin) which cleave a polypeptide at specific sites or digest the peptides from the amino or carboxy termini (e.g. diaminopeptidase) of the peptide chain are known. Furthermore, particular chemicals (e.g. cyanogen bromide) cleave a polypeptide chain at specific sites. The skilled artisan will appreciate the modifications necessary to the amino acid sequence (and synthetic or semisynthetic coding sequence if recombinant means are employed) to incorporate site-specific internal cleavage sites. See e.g., P. Carter, "Site Specific Proteolysis of Fusion Proteins", Chapter 13, in *Protein Purification: From Molecular Mechanisms to Large Scale Processes*, American Chemical Society, Washington, D.C. (1990).

[0038] The present invention contemplates ECB binding fusion proteins comprising a fragment of glucan synthase in fusion with another protein, thereby facilitating isolation, purification, and assay of said ECB binding fragment. A variety of embodiments and methods for producing fusion proteins are known in the art and are suitable for the present invention. For example, foreign proteins may be fused with the carboxy terminus of Sj26, a 26 kDa glutathione S-transferase (GST), encoded by the parasitic helminth *Schistosoma japonicum*. Such fusion proteins may be expressed in *E. coli* or other suitable procaryote, or in eucaryotic hosts, such as yeast. In this regard, the method and vectors of Smith and Johnson are especially suitable (*Gene*, 67, 31-40, 1988), the entire contents of which is incorporated by reference. It is desirable that the fusion protein remain in solution to facilitate ease of purification.

[0039] In addition to procaryotes, a variety of mammalian cell systems and eucaryotic microorganisms such as yeast

are suitable host cells for the recombinant expression of proteins or fusion proteins. The yeast *Saccharomyces cerevisiae* is the most commonly used eucaryotic microorganism. A number of other yeasts such as *Kluyveromyces lactis* and *Schizosaccharomyces pombe* are also suitable. For expression in *Saccharomyces*, the plasmid YRp7 (ATCC-40053), for example, may be used. See, e.g., D. Stinchcomb, *et al.*, *Nature*, 282:39 (1979); J. Kingsman *et al.*, *Gene*, 7:141 (1979); S. Tschemper *et al.*, *Gene*, 10:157 (1980). Plasmid YRp7 contains the TRP1 gene which provides a selectable marker for use in a trp1 auxotrophic mutant. For expression in *S. pombe* suitable vectors include those containing the *nmt1* promoter as well as the *adh* promoter and the SV40 promoter (See e.g. S. Forsburg, *Nuc. Acid. Res.* 21, 2955, 1993).

10 Purification of Recombinantly-Produced ECB Binding Peptide

[0040] An expression vector comprising a cloned nucleic acid encoding an ECB binding domain is transformed or transfected into a suitable host cell using standard methods. Cells that contain the vector are propagated under conditions suitable for expression of the peptide. If the gene is controlled by an inducible promoter, suitable growth conditions should incorporate the appropriate inducer. Recombinantly-produced peptide may be purified from cellular extracts of transformed cells by any suitable means. In one process for peptide purification, the gene is modified at the 5' end to incorporate several histidine residues at the amino terminus of the peptide. This "histidine tag" enables a single-step protein purification method referred to as "immobilized metal ion affinity chromatography" (IMAC), essentially as described in U.S. Patent 4,569,794 which hereby is incorporated by reference. The IMAC method enables rapid isolation of substantially pure peptide starting from a crude cellular extract.

[0041] Other embodiments of the present invention comprise isolated nucleic acid sequences that comprise SEQ ID NO:2, wherein said sequences encode amino acid residues 583 to 672 of SEQ ID NO:2. As skilled artisans will recognize, the amino acid compounds of the invention can be encoded by a multitude of different nucleic acid sequences because most of the amino acids are encoded by more than one codon due to the degeneracy of the genetic code. Because these alternative nucleic acid sequences would encode the same amino acid sequences, the present invention further comprises these alternate nucleic acid sequences.

[0042] Nucleic acids encoding an ECB binding domain of SEQ ID NO:2 may be produced by synthetic methods. Fragments of the proteins disclosed herein may be generated by any number of suitable techniques, including chemical synthesis of a suitable portion of SEQ ID NO:2, proteolytic digestion of SEQ ID NO:2, or most preferably, by recombinant DNA mutagenesis techniques, well known to the skilled artisan. See, e.g. K. Struhl, "Reverse biochemistry: Methods and applications for synthesizing yeast proteins *in vitro*," *Meth. Enzymol.* 194, 520-535. For example, in a preferred method, a nested set of deletion mutations are introduced into the intact *FKS1* gene (SEQ ID NO:1) encoding the native glucan synthase protein, such that varying amounts of the protein coding region are deleted, either from the amino terminal end, or from the carboxyl end of the protein molecule, and wherein said deletions produce molecules that retain amino acid residues from about 605 to 650, or more preferably amino acid residues from about 583 to 672 of SEQ ID NO:2. Internal fragments of the intact protein can also be produced in which both the carboxyl and amino terminal ends are removed. Several nucleases can be used to generate deletions, for example *Bal* 31, or in the case of a single stranded nucleic acid molecule, mung bean nuclease. For simplicity, it is preferred that the intact *FKS1* gene be cloned into a single-stranded cloning vector, such as bacteriophage M13, or equivalent. If desired, the resulting gene deletion fragments can be subcloned into any suitable vector for propagation and expression of said fragments in any suitable host cell. It is preferred that the fragments be subcloned into a plasmid, for example pGEX-1 (Smith & Johnson, *Gene*, 67, 31, 1988), enabling the production of a fusion protein comprising an ECB binding domain

[0043] The present invention provides fragments of the intact glucan synthase protein disclosed herein wherein said fragments retain the ability to bind ECB or other echinocandin or papulocandin.

[0044] ECB binding fragments of the intact proteins disclosed herein may be produced as described above, preferably using cloning techniques to produce fragments of the intact *FKS1* gene. Peptide fragments of glucan synthase or fusion proteins comprising a peptide fragment of glucan synthase may be tested for binding activity using any suitable assay.

[0045] The synthesis of nucleic acids is well known in the art. See, e.g., E.L. Brown, R. Belagaje, M.J. Ryan, and H. G. Khorana, *Methods in Enzymology*, 68:109-151 (1979). The nucleic acids of this invention could be generated using a conventional DNA synthesizing apparatus, such as the Applied Biosystems Model 380A or 380B DNA synthesizers (Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404) which employ phosphoramidite chemistry. Alternatively, phosphotriester chemistry may be employed to synthesize the nucleic acids of this invention. [See, e.g., M.J. Gait, ed., *Oligonucleotide Synthesis, A Practical Approach*, (1984).]

[0046] In an alternative methodology, namely PCR, the nucleic acids comprising a portion or all of SEQ ID NO:1 can be generated from *S. cerevisiae* genomic DNA using suitable oligonucleotide primers complementary to SEQ ID NO:1 or region therein, as described in U.S. Patent No. 4,889,818, which hereby is incorporated by reference. Suitable protocols for performing the PCR are disclosed in, for example, PCR Protocols: A Guide to Method and Applications, Ed. Michael A. Innis et al., Academic Press, Inc. (1990).

[0047] The ribonucleic acids of the present invention may be prepared using the polynucleotide synthetic methods discussed *supra*, or they may be prepared enzymatically using RNA polymerase to transcribe a DNA template.

[0048] The most preferred systems for preparing the ribonucleic acids of the present invention employ the RNA polymerase from the bacteriophage T7 or the bacteriophage SP6. These RNA polymerases are highly specific, requiring the insertion of bacteriophage-specific sequences at the 5' end of the template to be transcribed. See, J. Sambrook, *et al.*, *supra*, at 18.82-18.84.

[0049] This invention also provides nucleic acids, RNA or DNA, which are complementary to the nucleic acids encoding the ECB binding domain of SEQ ID NO:2.

[0050] The present invention also provides probes and primers useful for a variety of molecular biology techniques including, for example, hybridization screens of genomic or subgenomic libraries. A nucleic acid compound comprising SEQ ID NO:1, or a complementary sequence thereof, or a fragment thereof, and which is at least 18 base pairs in length, and which will selectively hybridize to *Saccharomyces cerevisiae* DNA or mRNA encoding *FKS1*, is provided. Preferably, the 18 or more base pair compound is DNA. A probe or primer length of at least 18 base pairs is dictated by theoretical and practical considerations. See e.g. B. Wallace and G. Miyada,

"Oligonucleotide Probes for the Screening of Recombinant DNA Libraries," In *Methods in Enzymology*, Vol. 152, 432-442, Academic Press (1987).

[0051] These probes and primers can be prepared by enzymatic methods well known to those skilled in the art (See e.g. Sambrook *et al. supra*). In a most preferred embodiment these probes and primers are synthesized using chemical means as described above.

[0052] Another aspect of the present invention relates to recombinant DNA cloning vectors and expression vectors comprising the nucleic acids of the present invention. Many of the vectors encompassed within this invention are described above. The preferred nucleic acid vectors are those which comprise DNA. The most preferred recombinant DNA vectors comprise nucleic acid encoding the ECB binding domain of SEQ ID NO:2.

[0053] The skilled artisan understands that choosing the most appropriate cloning vector or expression vector depends upon a number of factors including the availability of restriction enzyme sites, the type of host cell into which the vector is to be transfected or transformed, the purpose of the transfection or transformation (e.g., stable transformation as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily assayable or selectable markers (e.g., antibiotic resistance and metabolic markers of one type and another), and the number of copies of the gene to be present in the host cell.

[0054] Vectors suitable to carry the nucleic acids of the present invention comprise RNA viruses, DNA viruses, lytic bacteriophages, lysogenic bacteriophages, stable bacteriophages, plasmids, viroids, and the like. The most preferred vectors are plasmids.

[0055] When preparing an expression vector the skilled artisan understands that there are many variables to be considered, for example, whether to use a constitutive or inducible promoter. Inducible promoters are preferred because they enable high level, regulatable expression of an operably linked gene. The skilled artisan will recognize a number of inducible promoters which respond to a variety of inducers, for example, carbon source, metal ions, heat, and others. The practitioner also understands that the amount of nucleic acid or protein to be produced dictates, in part, the selection of the expression system. The addition of certain nucleotide sequences is useful for directing the localization of a recombinant protein. For example, a sequence encoding a signal peptide preceding the coding region of a gene, is useful for directing the extra-cellular export of a resulting polypeptide.

[0056] The present invention also provides a method for constructing a recombinant host cell capable of expressing the ECB binding domain of SEQ ID NO:2, said method comprising transforming or otherwise introducing into a host cell a recombinant DNA vector that comprises an isolated DNA sequence encoding amino acid residues from about 583 to 672 of SEQ ID NO:2. Suitable host cells include any strain of *E. coli* or *S. cerevisiae* that can accommodate high level expression of an exogenously introduced gene. Transformed host cells may be cultured under conditions well known to skilled artisans such that the ECB binding domain is expressed, thereby producing ECB binding peptide in the recombinant host cell.

[0057] Agents that bind the ECB binding domain may identify new antifungal compounds. Substances that bind the ECB binding peptide can be identified by contacting the peptide with a test compound and monitoring the interaction by any suitable means.

[0058] The instant invention provides a screening method for discovering compounds that bind the ECB binding peptide, said method comprising the steps of:

- a) preparing the binding peptide, preferably as a fusion protein;
- b) exposing said peptide or protein to a test compound; and
- c) quantifying the binding of said compound to said peptide by any suitable means.

[0059] In one embodiment, a protein comprising a fusion of the 89 amino acid residue ECB binding domain of SEQ ID NO:2 and a GST protein is expressed in yeast or *E. coli*, and purified for use in a microtiter plate ELISA screen. The ELISA screen enables an assay for the displacement of ECB from the ECB binding domain by a test compound. Bound ECB, or ECB free in solution can be detected using an ECB-specific antibody prepared using standard methods. If a test compound displaces ECB from the binding domain there will be a diminution in the ELISA signal. This method involves coating the wells of a microtiter plate with, for example, a GST-FKS1 fusion protein. After blocking residual binding sites the plates are rinsed to remove unbound fusion protein and then incubated with ECB. After rinsing again to remove unbound ECB, a test compound is added, incubated, and rinsed to remove unbound test compound or displaced ECB. The plates are then incubated with an antibody against ECB that is covalently linked to alkaline phosphatase (anti-ECB-AP). The plates are developed by adding an appropriate substrate, e.g. p-nitrophenyl phosphate for colorimetric detection, or 4-methylumbelliferyl phosphate for fluorimetric detection.

[0060] This screening method may be adapted to automated procedures such as a PANDEX® (Baxter-Dade Diagnostics) system, allowing for efficient high-volume screening of potential therapeutic agents.

[0061] In such a screening protocol an ECB binding peptide is prepared as described herein, preferably using recombinant DNA technology. A test compound is introduced into the reaction vessel containing the peptide.

[0062] Skilled artisans will recognize that IC₅₀ values are dependent on the selectivity of the compound tested. For example, a compound with an IC₅₀ which is less than 10 nM is generally considered an excellent candidate for drug therapy. However, a compound which has a lower affinity, but is selective for a particular target, may be an even better candidate. The skilled artisan will recognize that any information regarding inhibitory activity or selectivity of a particular compound is beneficial in the pharmaceutical arts.

[0063] The following examples more fully describe the present invention. Those skilled in the art will recognize that the particular reagents, equipment, and procedures described are merely illustrative and are not intended to limit the present invention in any manner.

EXAMPLE 1

Expression Vector Encoding the ECB Binding Domain

[0064] A vector for expressing a fusion protein in yeast comprising the ECB binding domain of yeast glucan synthase and glutathione S-transferase (GST) is prepared as follows. Plasmid pGEX-1 (Smith and Johnson, Gene, 67, 31-40, 1988) is an *E. coli* expression vector that comprises the *tac* promoter and the complete coding sequence of Sjl26 (viz. GST), in which the normal termination codon is replaced by a polylinker containing unique BamH1, Sma1, and EcoR1 restriction sites, followed by a termination codon in all 3 reading frames. A fragment of pGEX-1 containing the described GST gene is isolated by any suitable subcloning method, well known to the skilled artisan. It is convenient, but not necessary, for subsequent cloning steps, to attach to the fragment containing the GST gene of pGEX-1 oligonucleotides containing specific restriction enzyme sites. For convenience, the GST fragment thus described is cloned into the multiple cloning site of yeast expression vector pREP1 (K. Maundrell, *J. Biol. Chem.* 265, 10857, 1990), in the correct orientation, downstream of the LEU2 gene, and *nmf1* promoter. pREP1 also contains an ARS element for replication in the host yeast. The resulting plasmid, pREP1-GST, is linearized at any one or more of BamH1, Sma1, or EcoR1 sites at the 3' end of the GST fragment, for cloning in the ECB binding domain.

[0065] A DNA fragment encoding the ECB binding domain of SEQ ID NO:2 is conveniently prepared by PCR. Oligonucleotide primers are prepared for priming DNA synthesis on opposite strands from nucleotide positions 1747 through 2016 of SEQ ID NO:1. It is convenient to include suitable restriction sites at the appropriate 5' or 3' end of the PCR primers for subsequent cloning. The ECB binding fragment so prepared is purified by any suitable method, for example, isolation by gel electrophoresis. The purified ECB binding fragment is ligated into pREP1-GST so that the ECB binding fragment is linked to the 3' end of the GST gene. This construct, pREP1-GST-ECB, produces a fusion protein comprising a GST-ECB binding domain.

EXAMPLE 2

E. coli Expression Vector Encoding the ECB Binding Domain

[0066] A vector for expressing a fusion protein in *E. coli* comprising the ECB binding domain of yeast glucan synthase and glutathione S-transferase (GST) is prepared as follows. Plasmid pGEX-1 (Smith and Johnson, Gene, 67, 31-40, 1988) is an *E. coli* expression vector that comprises the *tac* promoter and the complete coding sequence of Sjl26 (viz. GST), in which the normal termination codon is replaced by a polylinker containing unique BamH1, Sma1, and EcoR1 restriction sites, followed by a termination codon in all 3 reading frames.

[0067] A DNA fragment encoding the ECB binding domain of SEQ ID NO:2 is conveniently prepared by PCR. Oli-

gonucleotide primers are prepared for priming DNA synthesis on opposite strands, from nucleotide positions 1747 through 2016 of SEQ ID NO:1. It is convenient to design into the oligonucleotide sequence suitable restriction sites at the termini for subsequent cloning steps. The ECB binding fragment so prepared is purified by any suitable method, for example, isolation from a gel following electrophoresis. The purified ECB binding fragment is ligated into pGEX-1 so that the ECB binding fragment is linked to the 3' end of the GST gene. This construct, pGST-ECB, produces a fusion protein comprising a GST-ECB binding domain.

EXAMPLE 3

Expression of ECB Fusion Protein in *S. pombe*

[0068] Expression plasmid pREP1-GST-ECB (Example 1) is transformed into any suitable strain of *S. pombe*, for example, a leu⁻ strain (See e.g. R. Sikorski & P. Hieter, *Genetics*, 122, 19-26, 1989; K. Maundrell, *J. Biol. Chem.* 265, 10857, 1990) using standard methods, for example, spheroplast transformation, or lithium acetate transformation (See e.g. Sambrook *et al. Supra*; Okazaki *et al. Nuc. Acid Res.* 18, 6485-89 (1990); Moreno *et al. Meth. Enzym.* 194, 795-823 (1991). Transformants, chosen at random, are tested for the presence of the plasmid by agarose gel electrophoresis using quick plasmid preparations. *Id.* Transformants are grown overnight under conditions suitable to induce the *nmt1* promoter, for example, in minimal medium lacking thiamine (Beach & Nurse, *Nature*, 290, 140, 1981). The overnight culture was diluted into fresh medium and allowed to grow to mid-log phase. The induced-culture was pelleted by centrifugation in preparation for protein purification.

EXAMPLE 4

Affinity Purification of a Recombinantly-Produced ECB Binding Domain

[0069] Overnight cultures of transformed *E. coli* or yeast cells, (See e.g. Example 3), are lysed by sonication with glass beads, or by spheroplast formation in MTPBS (150 mM NaCl, 16 mM Na₂HPO₄, 4 mM NaH₂PO₄ (pH 7.3) and including 1% Triton X-100 (BDH Chemicals). Lysed cells are subjected to centrifugation at 10,000 x g for 5 minutes at 4° C. The supernatant is mixed on a rotating platform with 1 to 2 ml 50% glutathione-agarose beads (sulphur linkage, Sigma). After absorption for 2 minutes, beads are collected by brief centrifugation at 500 x g and washed 3 times with 50 ml MTPBS. Fusion protein is eluted by competition with free glutathione, using 2 x 2 minute washes with 1 bead volume of 50 mM Tris HCl, pH 8, containing 5 mM reduced glutathione (Sigma), pH 7.5.

Annex to the description

[0070]

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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- (i) APPLICANT: ELI LILLY AND COMPANY
 (B) STREET: Lilly Corporate Center
 (C) CITY: Indianapolis
 (D) STATE: Indiana
 (E) COUNTRY: United States of America
 (F) ZIP: 46285

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- (ii) TITLE OF INVENTION: Echinocandin Binding Site of
 1,3-B-Glucan Synthase

- (iii) NUMBER OF SEQUENCES: 2

20

- (iv) CORRESPONDENCE ADDRESS:
 (A) ADDRESSEE: A. M. Denholm
 (B) STREET: Erl Wood Manor
 (C) CITY: Windlesham
 (D) STATE: Surrey
 (E) COUNTRY: United Kingdom
 (F) ZIP: GU20 6PH

25

- (v) COMPUTER READABLE FORM:
 (A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5631 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

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- (iv) ANTI-SENSE: NO

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..5628

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- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG AAC ACT GAT CAA CAA CCT TAT CAG GGC CAA ACG GAC TAT ACC CAG 48
 Met Asn Thr Asp Gln Gln Pro Tyr Gln Gly Gln Thr Asp Tyr Thr Gln
 1 5 10 15

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GGA CCA GGT AAC GGG CAA AGT CAG GAA CAA GAC TAT GAC CAA TAT GGC 96
 Gly Pro Gly Asn Gly Gln Ser Gln Glu Gln Asp Tyr Asp Gln Tyr Gly
 20 25 30

CAG CCT TTG TAT CCT TCA CAA GCT GAT GGT TAC TAC GAT CCA AAT GTC 144
 Gln Pro Leu Tyr Pro Ser Gln Ala Asp Gly Tyr Tyr Asp Pro Asn Val
 35 40 45

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	GCT GCT GGT ACT GAA GCT GAT ATG TAT GGT CAA CAA CCA CCA AAC GAG	192
	Ala Ala Gly Thr Glu Ala Asp Met Tyr Gly Gln Gln Pro Pro Asn Glu	
5	50 55 60	
	TCT TAC GAC CAA GAC TAC ACA AAC GGT GAA TAC TAT GGT CAA CCG CCA	240
	Ser Tyr Asp Gln Asp Tyr Thr Asn Gly Glu Tyr Tyr Gly Gln Pro Pro	
	65 70 75 80	
	AAT ATG GCT GCT CAA GAC GGT GAA AAC TTC TCG GAT TTT AGC AGT TAC	288
10	Asn Met Ala Ala Gln Asp Gly Glu Asn Phe Ser Asp Phe Ser Ser Tyr	
	85 90 95	
	GGC CCT CCT GGA ACA CCT GGA TAT GAT AGC TAT GGT GGT CAG TAT ACC	336
	Gly Pro Pro Gly Thr Pro Gly Tyr Ser Tyr Gly Gly Gln Tyr Thr	
	100 105 110	
15	GCT TCT CAA ATG AGT TAT GGA GAA CCA AAT TCG TCG GGT ACC TCG ACT	384
	Ala Ser Gln Met Ser Tyr Gly Glu Pro Asn Ser Ser Gly Thr Ser Thr	
	115 120 125	
	CCA ATT TAC GGT AAT TAT GAC CCA AAT GCT ATC GCT ATG GCT TTG CCA	432
20	Pro Ile Tyr Gly Asn Tyr Asp Pro Asn Ala Ile Ala Met Ala Leu Pro	
	130 135 140	
	AAT GAA CCT TAT CCC GCT TGG ACT GCT GAC TCT CAA TCT CCC GTT TCG	480
	Asn Glu Pro Tyr Pro Ala Trp Thr Ala Asp Ser Gln Ser Pro Val Ser	
	145 150 155 160	
	ATC GAG CAA ATC GAA GAT ATC TTT ATT GAT TTG ACC AAC AGA CTC GGG	528
25	Ile Glu Gln Ile Glu Asp Ile Phe Ile Asp Leu Thr Asn Arg Leu Gly	
	165 170 175	
	TTC CAA AGA GAC TCC ATG AGA AAT ATG TTT GAT CAT TTT ATG GTT CTC	576
	Phe Gln Arg Asp Ser Met Arg Asn Met Phe Asp His Phe Met Val Leu	
	180 185 190	
30	TTG GAC TCT AGG TCC TCG AGA ATG TCT CCT GAT CAA GCT TTA CTA TCT	624
	Leu Asp Ser Arg Ser Ser Arg Met Ser Pro Asp Gln Ala Leu Leu Ser	
	195 200 205	
	TTA CAT GCC GAC TAC ATT GGT GGC GAT ACT GCT AAC TAT AAA AAA TGG	672
35	Leu His Ala Asp Tyr Ile Gly Gly Asp Thr Ala Asn Tyr Lys Lys Trp	
	210 215 220	
	TAT TTT GCT GCT CAG TTA GAT ATG GAT GAT GAA ATT GGT TTT AGA AAT	720
	Tyr Phe Ala Ala Gln Leu Asp Met Asp Asp Glu Ile Gly Phe Arg Asn	
	225 230 235 240	
	ATG AGT CTT GGA AAA CTC TCA AGG AAG GCA AGA AAA GCT AAG AAG AAA	768
40	Met Ser Leu Gly Lys Leu Ser Arg Lys Ala Arg Lys Ala Lys Lys Lys	
	245 250 255	
	AAC AAG AAA GCA ATG GAA GAG GCC AAT CCC GAA GAC ACT GAA GAA ACT	816
	Asn Lys Lys Ala Met Glu Glu Ala Asn Pro Glu Asp Thr Glu Glu Thr	
	260 265 270	
45	TTA AAC AAA ATT GAA GGC GAC AAC TCC CTA GAG GCT GCT GAT TTT AGA	864
	Leu Asn Lys Ile Glu Gly Asp Asn Ser Leu Glu Ala Ala Asp Phe Arg	
	275 280 285	
	TGG AAG GCC AAG ATG AAC CAG TTG TCT CCC CTG GAA AGA GTT CGT CAT	912
50	Trp Lys Ala Lys Met Asn Gln Leu Ser Pro Leu Glu Arg Val Arg His	
	290 295 300	
55		

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5	ATC GCC TTA TAT CTG TTA TGT TGG GGT GAA GCT AAT CAA GTC AGA TTC Ile Ala Leu Tyr Leu Leu Cys Trp Gly Glu Ala Asn Gln Val Arg Phe 305 310 315 320	960
	ACT GCT GAA TGT TTA TGT TTT ATC TAC AAG TGT GCT CTT GAC TAC TTG Thr Ala Glu Cys Leu Cys Phe Ile Tyr Lys Cys Ala Leu Asp Tyr Leu 325 330 335	1008
	GAT TCC CCT CTT TGC CAA CAA CGC CAA GAA CCT ATG CCA GAA GGT GAT Asp Ser Pro Leu Cys Gln Gln Arg Gln Glu Pro Met Pro Glu Gly Asp 340 345 350	1056
	TTC TTG AAT AGA GTC ATT ACG CCA ATT TAT CAT TTC ATC AGA AAT CAA Phe Leu Asn Arg Val Ile Thr Pro Ile Tyr His Phe Arg Asn Gln 355 360 365	1104
	GTT TAT GAA ATT GTT GAT GGT CGT TTT GTC AAG CGT GAA AGA GAT CAT Val Tyr Glu Ile Val Asp Gly Arg Phe Val Lys Arg Glu Arg Asp His 370 375 380	1152
20	AAC AAA ATT GTC GGT TAT GAT GAT TTA AAC CAA TTG TTC TGG TAT CCA Asn Lys Ile Val Gly Tyr Asp Asp Leu Asn Gln Leu Phe Trp Tyr Pro 385 390 395 400	1200
	GAA GGT ATT GCA AAG ATT GTT CTT GAA GAT GGA ACA AAA TTG ATA GAA Glu Gly Ile Ala Lys Ile Val Leu Glu Asp Gly Thr Lys Leu Ile Glu 405 410 415	1248
25	CTC CCA TTG GAA GAA CGT TAT TTA AGA TTA GGC GAT GTC GTC TGG GAT Leu Pro Leu Glu Glu Arg Tyr Leu Arg Leu Gly Asp Val Val Trp Asp 420 425 430	1296
	GAT GTA TTC TTC AAA ACA TAT AAA GAG ACC CGT ACT TGG TTA CAT TTG Asp Val Phe Phe Lys Thr Tyr Lys Glu Thr Arg Thr Trp Leu His Leu 435 440 445	1344
30	GTC ACC AAC TTC AAC CGT ATT TGG GTT ATG CAT ATC TCC ATT TTT TGG Val Thr Asn Phe Asn Arg Ile Trp Val Met His Ile Ser Ile Phe Trp 450 455 460	1392
	ATG TAC TTT GCA TAT AAT TCA CCA ACA TTT TAC ACT CAT AAC TAT CAA Met Tyr Phe Ala Tyr Asn Ser Pro Thr Phe Tyr Thr His Asn Tyr Gln 465 470 475 480	1440
35	CAA TTG GTC GAC AAC CAA CCT TTG GCT GCT TAC AAG TGG GCA TCT TGC Gln Leu Val Asp Asn Gln Pro Leu Ala Ala Tyr Lys Trp Ala Ser Cys 485 490 495	1488
	GCA TTA GGT GGT ACT GTC GCA AGT TTG ATT CAA ATT GTC GCT ACT TTG Ala Leu Gly Gly Thr Val Ala Ser Leu Ile Gln Ile Val Ala Thr Leu 500 505 510	1536
40	TGT GAA TGG TCA TTC GTT CCA AGA AAA TGG GCT GGT GCT CAA CAT CTA Cys Glu Trp Ser Phe Val Pro Arg Lys Trp Ala Gly Ala Gln His Leu 515 520 525	1584
	TCT CGT AGA TTC TGG TTT TTA TGC ATC ATC TTT GGT ATT AAT TTG GGT Ser Arg Arg Phe Trp Phe Leu Cys Ile Ile Phe Gly Ile Asn Leu Gly 530 535 540	1632
45	CCT ATT ATT TTT GTT TTT GCT TAC GAC AAA GAT ACA GTC TAC TCC ACT Pro Ile Ile Phe Val Phe Ala Tyr Asp Lys Asp Thr Val Tyr Ser Thr 545 550 555 560	1680
	GCT GCA CAC GTT GTT GCT GCT GTT ATG TTC TTT GTT GCG GTT GCT ACC	1728

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	Ala	Ala	His	Val	Val	Ala	Ala	Val	Met	Phe	Phe	Val	Ala	Val	Ala	Thr	
					565					570					575		
5	ATC	ATA	TTC	TTC	TCC	ATT	ATG	CCA	TTG	GGG	GGG	TTG	TTT	ACG	TCA	TAT	1776
	Ile	Ile	Phe	Phe	Ser	Ile	Met	Pro	Leu	Gly	Gly	Leu	Phe	Thr	Ser	Tyr	
				580					585					590			
	ATG	AAA	AAA	TCT	ACA	AGG	CGT	TAT	GTT	GCA	TCT	CAA	ACA	TTC	ACT	GCT	1824
	Met	Lys	Lys	Ser	Thr	Arg	Arg	Tyr	Val	Ala	Ser	Gln	Thr	Phe	Thr	Ala	
			595					600					605				
10	GCA	TTT	GCC	CCT	CTA	CAT	GGG	TTA	GAT	AGA	TGG	ATG	TCC	TAT	TTA	GTT	1872
	Ala	Phe	Ala	Pro	Leu	His	Gly	Leu	Asp	Arg	Trp	Met	Ser	Tyr	Leu	Val	
		610					615					620					
	TGG	GTT	ACT	GTT	TTT	GCT	GCC	AAA	TAT	TCA	GAA	TCG	TAC	TAC	TTT	TTA	1920
15	Trp	Val	Thr	Val	Phe	Ala	Ala	Lys	Tyr	Ser	Glu	Ser	Tyr	Tyr	Phe	Leu	
		625				630					635					640	
	GTT	TTA	TCT	TTG	AGA	GAT	CCA	ATT	AGA	ATT	TTG	TCC	ACC	ACT	GCA	ATG	1968
	Val	Leu	Ser	Leu	Arg	Asp	Pro	Ile	Arg	Ile	Leu	Ser	Thr	Thr	Ala	Met	
					645					650					655		
20	AGG	TGT	ACA	GGT	GAA	TAC	TGG	TGG	GGT	GCG	GTA	CTT	TGT	AAA	GTG	CAA	2016
	Arg	Cys	Thr	Gly	Glu	Tyr	Trp	Trp	Gly	Ala	Val	Leu	Cys	Lys	Val	Gln	
				660					665					670			
	CCC	AAG	ATT	GTC	TTA	GGT	TTG	GTT	ATC	GCT	ACC	GAC	TTC	ATT	CTT	TTC	2064
	Pro	Lys	Ile	Val	Leu	Gly	Leu	Val	Ile	Ala	Thr	Asp	Phe	Ile	Leu	Phe	
			675					680					685				
25	TTC	TTG	GAT	ACC	TAC	TTA	TGG	TAC	ATT	ATT	GTG	AAT	ACC	ATT	TTC	TCT	2112
	Phe	Leu	Asp	Thr	Tyr	Leu	Trp	Tyr	Ile	Ile	Val	Asn	Thr	Ile	Phe	Ser	
		690					695					700					
	GTT	GGG	AAA	TCT	TTC	TAT	TTA	GGT	ATT	TCT	ATC	TTA	ACA	CCA	TGG	AGA	2160
30	Val	Gly	Lys	Ser	Phe	Tyr	Leu	Gly	Ile	Ser	Ile	Leu	Thr	Pro	Trp	Arg	
		705				710					715					720	
	AAT	ATC	TTC	ACA	AGA	TTG	CCA	AAA	AGA	ATA	TAC	TCC	AAG	ATT	TTG	GCT	2208
	Asn	Ile	Phe	Thr	Arg	Leu	Pro	Lys	Arg	Ile	Tyr	Ser	Lys	Ile	Leu	Ala	
					725					730				735			
35	ACT	ACT	GAT	ATG	GAA	ATT	AAA	TAC	AAA	CCA	AAG	GTT	TTG	ATT	TCT	CAA	2256
	Thr	Thr	Asp	Met	Glu	Ile	Lys	Tyr	Lys	Pro	Lys	Val	Leu	Ile	Ser	Gln	
				740				745					750				
	GTA	TGG	AAT	GCC	ATC	ATT	ATT	TCA	ATG	TAC	AGA	GAA	CAT	CTC	TTA	GCC	2304
	Val	Trp	Asn	Ala	Ile	Ile	Ile	Ser	Met	Tyr	Arg	Glu	His	Leu	Leu	Ala	
			755					760					765				
40	ATC	GAC	CAT	GTA	CAA	AAA	TTA	CTA	TAT	CAT	CAA	GTT	CCA	TCT	GAA	ATC	2352
	Ile	Asp	His	Val	Gln	Lys	Leu	Leu	Tyr	His	Gln	Val	Pro	Ser	Glu	Ile	
		770					775					780					
	GAA	GGT	AAA	AGA	ACT	TTG	AGA	GCT	CCT	ACC	TTC	TTT	GTT	TCT	CAA	GAT	2400
45	Glu	Gly	Lys	Arg	Thr	Leu	Arg	Ala	Pro	Thr	Phe	Phe	Val	Ser	Gln	Asp	
		785				790					795					800	
	GAC	AAT	AAT	TTT	GAG	ACT	GAA	TTT	TTC	CCT	AGG	GAT	TCA	GAG	GCT	GAG	2448
	Asp	Asn	Asn	Phe	Glu	Thr	Glu	Phe	Phe	Pro	Arg	Asp	Ser	Glu	Ala	Glu	
				805						810					815		
50	CGT	CGT	ATT	TCT	TTC	TTT	GCT	CAA	TCT	TTG	TCT	ACT	CCA	ATT	CCC	GAA	2496
	Arg	Arg	Ile	Ser	Phe	Phe	Ala	Gln	Ser	Leu	Ser	Thr	Pro	Ile	Pro	Glu	
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	820							825							830							
5	CCA	CTT	CCA	GTT	GAT	AAC	ATG	CCA	ACG	TTC	ACA	GTA	TTG	ACT	CCT	CAC	2544					
	Pro	Leu	Pro	Val	Asp	Asn	Met	Pro	Thr	Phe	Thr	Val	Leu	Thr	Pro	His						
			835					840					845									
	TAC	GCG	GAA	AGA	ATT	CTG	CTG	TCA	TTA	AGA	GAA	ATT	ATT	CGT	GAA	GAT	2592					
	Tyr	Ala	Glu	Arg	Ile	Leu	Leu	Ser	Leu	Arg	Glu	Ile	Ile	Arg	Glu	Asp						
		850				855						860										
10	GAC	CAA	TTT	TCT	AGA	GTT	ACT	CTT	TTA	GAA	TAT	CTA	AAA	CAA	TTA	CAT	2640					
	Asp	Gln	Phe	Ser	Arg	Val	Thr	Leu	Leu	Glu	Tyr	Leu	Lys	Gln	Leu	His						
	865				870						875				880							
	CCC	GTT	GAA	TGG	GAA	TGT	TTT	GTT	AAG	GAT	ACT	AAG	ATT	TTG	GCT	GAA	2688					
	Pro	Val	Glu	Trp	Glu	Cys	Phe	Val	Lys	Asp	Thr	Lys	Ile	Leu	Ala	Glu						
					885					890					895							
15	GAA	ACC	GCT	GCC	TAT	GAA	GGA	AAT	GAA	AAT	GAA	GCT	GAA	AAG	GAA	GAT	2736					
	Glu	Thr	Ala	Ala	Tyr	Glu	Gly	Asn	Glu	Asn	Glu	Ala	Glu	Lys	Glu	Asp						
				900				905					910									
	GCT	TTG	AAA	TCT	CAA	ATC	GAT	GAT	TTG	CCA	TTT	TAT	TGT	ATT	GGT	TTT	2784					
20	Ala	Leu	Lys	Ser	Gln	Ile	Asp	Asp	Leu	Pro	Phe	Tyr	Cys	Ile	Gly	Phe						
			915				920						925									
	AAA	TCT	GCT	GCT	CCA	GAA	TAT	ACA	CTT	CGT	ACG	AGA	ATT	TGG	GCT	TCT	2832					
	Lys	Ser	Ala	Ala	Pro	Glu	Tyr	Thr	Leu	Arg	Thr	Arg	Ile	Trp	Ala	Ser						
		930				935						940										
25	TTG	AGG	TCG	CAG	ACT	CTA	TAT	CGT	ACC	ATT	TCA	GGG	TTC	ATG	AAT	TAT	2880					
	Leu	Arg	Ser	Gln	Thr	Leu	Tyr	Arg	Thr	Ile	Ser	Gly	Phe	Met	Asn	Tyr						
		945				950					955					960						
	TCA	AGA	GCT	ATC	AAA	TTA	CTG	TAT	CGT	GTG	GAA	AAT	CCT	GAA	ATT	GTT	2928					
	Ser	Arg	Ala	Ile	Lys	Leu	Leu	Tyr	Arg	Val	Glu	Asn	Pro	Glu	Ile	Val						
					965					970					975							
30	CAA	ATG	TTT	GGT	GGT	AAT	GCT	GAA	GGC	TTA	GAA	AGA	GAG	CTA	GAA	AAG	2976					
	Gln	Met	Phe	Gly	Gly	Asn	Ala	Glu	Gly	Leu	Glu	Arg	Glu	Leu	Glu	Lys						
				980				985						990								
	ATG	GCA	AGA	AGA	AAG	TTT	AAA	TTT	TTG	GTC	TCT	ATG	CAG	AGA	TTG	GCT	3024					
35	Met	Ala	Arg	Arg	Lys	Phe	Lys	Phe	Leu	Val	Ser	Met	Gln	Arg	Leu	Ala						
			995				1000						1005									
	AAA	TTC	AAA	CCA	CAT	GAA	CTG	GAA	AAT	GCT	GAG	TTT	TTG	TTG	AGA	GCT	3072					
	Lys	Phe	Lys	Pro	His	Glu	Leu	Glu	Asn	Ala	Glu	Phe	Leu	Leu	Arg	Ala						
		1010				1015						1020										
40	TAC	CCA	GAC	TTA	CAA	ATT	GCC	TAC	TTG	GAT	GAA	GAG	CCA	CCT	TTG	ACT	3120					
	Tyr	Pro	Asp	Leu	Gln	Ile	Ala	Tyr	Leu	Asp	Glu	Glu	Pro	Pro	Leu	Thr						
		1025				1030					1035					1040						
	GAA	GGT	GAG	GAG	CCA	AGA	ATC	TAT	TCC	GCT	TTG	ATT	GAT	GGA	CAT	TGT	3168					
	Glu	Gly	Glu	Glu	Pro	Arg	Ile	Tyr	Ser	Ala	Leu	Ile	Asp	Gly	His	Cys						
					1045					1050					1055							
45	GAA	ATT	CTA	GAT	AAT	GGT	CGT	AGA	CGT	CCC	AAG	TTT	AGA	GTT	CAA	TTA	3216					
	Glu	Ile	Leu	Asp	Asn	Gly	Arg	Arg	Arg	Pro	Lys	Phe	Arg	Val	Gln	Leu						
				1060					1065					1070								
	TCT	GGT	AAC	CCA	ATT	CTT	GGT	GAC	GGT	AAA	TCT	GAT	AAC	CAA	AAC	CAT	3264					
50	Ser	Gly	Asn	Pro	Ile	Leu	Gly	Asp	Gly	Lys	Ser	Asp	Asn	Gln	Asn	His						
			1075				1080						1085									

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	GCT TTG ATT TTT TAC AGA GGT GAA TAC ATT CAA TTA ATT GAT GCC AAC	3312
	Ala Leu Ile Phe Tyr Arg Gly Glu Tyr Ile Gln Leu Ile Asp Ala Asn	
	109C 1095 1100	
5	CAA GAT AAC TAC TTG GAA GAA TGT CTG AAG ATT AGA TCT GTA TTG GCT	3360
	Gln Asp Asn Tyr Leu Glu Glu Cys Leu Lys Ile Arg Ser Val Leu Ala	
	1105 1110 1115 1120	
	GAA TTT GAG GAA TTG AAC GTT GAA CAA GTT AAT CCA TAT GCT CCC GGT	3408
10	Glu Phe Glu Glu Leu Asn Val Glu Gln Val Asn Pro Tyr Ala Pro Gly	
	1125 1130 1135	
	TTA AGG TAT GAG GAG CAA ACA ACT AAT CAT CCT GTT GCT ATT GTT GGT	3456
	Leu Arg Tyr Glu Glu Gln Thr Thr Asn His Pro Val Ala Ile Val Gly	
	1140 1145 1150	
15	GCC AGA GAA TAC ATT TTC TCT GAA AAC TCT GGT GTG CTG GGT GAT GTG	3504
	Ala Arg Glu Tyr Ile Phe Ser Glu Asn Ser Gly Val Leu Gly Asp Val	
	1155 1160 1165	
	GCC GCT GGT AAA GAA CAA ACT TTT GGT ACA TTA TTT GCG CGT ACT TTA	3552
	Ala Ala Gly Lys Glu Gln Thr Phe Gly Thr Leu Phe Ala Arg Thr Leu	
	1170 1175 1180	
20	TCT CAA ATT GGT GGT AAA TTG CAT TAT GGT CAT CCG GAT TTC ATT AAT	3600
	Ser Gln Ile Gly Gly Lys Leu His Tyr Gly His Pro Asp Phe Ile Asn	
	1185 1190 1195 1200	
	GCT ACG TTT ATG ACC ACT AGA GGT GGT GTT TCC AAA GCA CAA AAG GGT	3648
25	Ala Thr Phe Met Thr Thr Arg Gly Gly Val Ser Lys Ala Gln Lys Gly	
	1205 1210 1215	
	TTG CAT TTA AAC GAA GAT ATT TAT GCT GGT ATG AAT GCT ATG CTT CGT	3696
	Leu His Leu Asn Glu Asp Ile Tyr Ala Gly Met Asn Ala Met Leu Arg	
	1220 1225 1230	
30	GGT GGT CGT ATC AAG CAT TGT GAG TAT TAT CAA TGT GGT AAA GGT AGA	3744
	Gly Gly Arg Ile Lys His Cys Glu Tyr Tyr Gln Cys Gly Lys Gly Arg	
	1235 1240 1245	
	GAT TTG GGT TTC GGT ACA ATT CTA AAT TTC ACT ACT AAG ATT GGT GCT	3792
	Asp Leu Gly Phe Gly Thr Ile Leu Asn Phe Thr Thr Lys Ile Gly Ala	
	1250 1255 1260	
35	GGT ATG GGT GAA CAA ATG TTA TCT CGT GAA TAT TAT TAT CTG GGT ACC	3840
	Gly Met Gly Glu Gln Met Leu Ser Arg Glu Tyr Tyr Tyr Leu Gly Thr	
	1265 1270 1275 1280	
	CAA TTA CCA GTG GAC CGT TTC CTA ACA TTC TAT TAT GCC CAT CCT GGT	3888
40	Gln Leu Pro Val Asp Arg Phe Leu Thr Phe Tyr Tyr Ala His Pro Gly	
	1285 1290 1295	
	TTC CAT TTG AAC AAC TTG TTC ATT CAA TTA TCT TTG CAA ATG TTT ATG	3936
	Phe His Leu Asn Asn Leu Phe Ile Gln Leu Ser Leu Gln Met Phe Met	
	1300 1305 1310	
45	TTG ACT TTG GTG AAT TTA TCT TCC TTG GCC CAT GAA TCT ATT ATG TGT	3984
	Leu Thr Leu Val Asn Leu Ser Ser Leu Ala His Glu Ser Ile Met Cys	
	1315 1320 1325	
	ATT TAC GAT AGG AAC AAA CCA AAA ACA GAT GTT TTG GTT CCA ATT GGG	4032
	Ile Tyr Asp Arg Asn Lys Pro Lys Thr Asp Val Leu Val Pro Ile Gly	
	1330 1335 1340	
50		
55		

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5	TGT TAC AAC TTC CAA CCT GCG GTT GAT TGG GTG AGA CGT TAT ACA TTG Cys Tyr Asn Phe Gln Pro Ala Val Asp Trp Val Arg Arg Tyr Thr Leu 1345 1350 1355 1360	4080
10	TCT ATT TTC ATT GTT TTC TGG ATT GCC TTC GTT CCT ATT GTT GTT CAA Ser Ile Phe Ile Val Phe Trp Ile Ala Phe Val Pro Ile Val Val Gln 1365 1370 1375	4128
15	GAA CTA ATT GAA CGT GGT CTA TGG AAA GCC ACC CAA AGA TTT TTC TGC Glu Leu Ile Glu Arg Gly Leu Trp Lys Ala Thr Gln Arg Phe Phe Cys 1380 1385 1390	4176
20	CAC CTA TTA TCA TTA TCC CCT ATG TTC GAA GTG TTT GCG GGC CAA ATC His Leu Leu Ser Leu Ser Pro Met Phe Glu Val Phe Ala Gly Gln Ile 1395 1400 1405	4224
25	TAC TCT TCT GCG TTA TTA AGT GAT TTA GCA ATT GGT GGT GCT CGT TAT Tyr Ser Ser Ala Leu Leu Ser Asp Leu Ala Ile Gly Gly Ala Arg Tyr 1410 1415 1420	4272
30	ATA TCC ACC GGT CGT GGT TTT GCA ACT TCT CGT ATA CCA TTT TCA ATT Ile Ser Thr Gly Arg Gly Phe Ala Thr Ser Arg Ile Pro Phe Ser Ile 1425 1430 1435 1440	4320
35	TTG TAT TCA AGA TTT GCA GGA TCT GCT ATC TAC ATG GGT GCA AGA TCA Leu Tyr Ser Arg Phe Ala Gly Ser Ala Ile Tyr Met Gly Ala Arg Ser 1445 1450 1455	4368
40	ATG TTA ATG TTG CTG TTC GGT ACT GTC GCA CAT TGG CAA GCT CCA CTA Met Leu Met Leu Leu Phe Gly Thr Val Ala His Trp Gln Ala Pro Leu 1460 1465 1470	4416
45	CTG TGG TTT TGG GCC TCT CTA TCT TCA TTA ATT TTT GCG CCT TTC GTT Leu Trp Phe Trp Ala Ser Leu Ser Ser Leu Ile Phe Ala Pro Phe Val 1475 1480 1485	4464
50	TTC AAT CCA CAT CAG TTT GCT TGG GAA GAT TTC TTT TTG GAT TAC AGG Phe Asn Pro His Gln Phe Ala Trp Glu Asp Phe Phe Leu Asp Tyr Arg 1490 1495 1500	4512
55	GAT TAT ATC AGA TGG TTA TCA AGA GGT AAT AAT CAA TAT CAT AGA AAC Asp Tyr Ile Arg Trp Leu Ser Arg Gly Asn Asn Gln Tyr His Arg Asn 1505 1510 1515 1520	4560
60	TCG TGG ATT GGT TAC GTG AGG ATG TCT AGG GCA CGT ATT ACT GGG TTT Ser Trp Ile Gly Tyr Val Arg Met Ser Arg Ala Arg Ile Thr Gly Phe 1525 1530 1535	4608
65	AAA CGT AAA CTG GTT GGC GAT GAA TCT GAG AAA GCT GCT GGT GAC GCA Lys Arg Lys Leu Val Gly Asp Glu Ser Glu Lys Ala Ala Gly Asp Ala 1540 1545 1550	4656
70	AGC AGG GCT CAT AGA ACC AAT TTG ATC ATG GCT GAA ATC ATA CCC TGT Ser Arg Ala His Arg Thr Asn Leu Ile Met Ala Glu Ile Ile Pro Cys 1555 1560 1565	4704
75	GCA ATT TAT GCA GCT GGT TGT TTT ATT GCC TTC ACG TTT ATT AAT GCT Ala Ile Tyr Ala Ala Gly Cys Phe Ile Ala Phe Thr Phe Ile Asn Ala 1570 1575 1580	4752
80	CAA ACC GGT GTC AAG ACT ACT GAT GAT GAT AGG GTG AAT TCT GTT TTA Gln Thr Gly Val Lys Thr Thr Asp Asp Asp Arg Val Asn Ser Val Leu 1585 1590 1595 1600	4800
85	CGT ATC ATC ATT TGT ACC TTG GCG CCA ATC GCC GTT AAC CTC GGT GTT	4848

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	Arg	Ile	Ile	Ile	Cys	Thr	Leu	Ala	Pro	Ile	Ala	Val	Asn	Leu	Gly	Val	
					1605					1610					1615		
5	CTA	TTC	TTC	TGT	ATG	GGT	ATG	TCA	TGC	TGC	TCT	GGT	CCC	TTA	TTT	GGT	4896
	Leu	Phe	Phe	Cys	Met	Gly	Met	Ser	Cys	Cys	Ser	Gly	Pro	Leu	Phe	Gly	
				1620					1625					1630			
	ATG	TGT	TGT	AAG	AAG	ACA	GGT	TCT	GTA	ATG	GCT	GGA	ATT	GCC	CAC	GGT	4944
	Met	Cys	Cys	Lys	Lys	Thr	Gly	Ser	Val	Met	Ala	Gly	Ile	Ala	His	Gly	
				1635			1640						1645				
10	GTT	GCT	GTT	ATT	GTC	CAC	ATT	GCC	TTT	TTC	ATT	GTC	ATG	TGG	GTT	TTG	4992
	Val	Ala	Val	Ile	Val	His	Ile	Ala	Phe	Phe	Ile	Val	Met	Trp	Val	Leu	
				1650			1655					1660					
	GAG	AGC	TTC	AAC	TTT	GTT	AGA	ATG	TTA	ATC	GGA	GTC	GTT	ACT	TGT	ATC	5040
15	Glu	Ser	Phe	Asn	Phe	Val	Arg	Met	Leu	Ile	Gly	Val	Val	Thr	Cys	Ile	
				1665			1670				1675					1680	
	CAA	TGT	CAA	AGA	CTC	ATT	TTT	CAT	TGC	ATG	ACA	GCG	TTA	ATG	TTG	ACT	5088
	Gln	Cys	Gln	Arg	Leu	Ile	Phe	His	Cys	Met	Thr	Ala	Leu	Met	Leu	Thr	
					1685				1690								
20	CGT	GAA	TTT	AAA	AAC	GAT	CAT	GCC	AAT	ACA	GCC	TTC	TGG	ACT	GGT	AAG	5136
	Arg	Glu	Phe	Lys	Asn	Asp	His	Ala	Asn	Thr	Ala	Phe	Trp	Thr	Gly	Lys	
				1700				1705						1710			
	TGG	TAT	GGT	AAA	GGT	ATG	GGT	TAC	ATG	GCT	TGG	ACC	CAG	CCA	AGT	AGA	5184
	Trp	Tyr	Gly	Lys	Gly	Met	Gly	Tyr	Met	Ala	Trp	Thr	Gln	Pro	Ser	Arg	
				1715			1720						1725				
25	GAA	TTA	ACC	GCC	AAG	GTA	ATT	GAG	CTT	TCA	GAA	TTT	GCA	GCT	GAT	TTT	5232
	Glu	Leu	Thr	Ala	Lys	Val	Ile	Glu	Leu	Ser	Glu	Phe	Ala	Ala	Asp	Phe	
				1730			1735					1740					
	GTT	CTA	GGT	CAT	GTG	ATT	TTA	ATC	TGT	CAA	CTG	CCA	CTC	ATT	ATA	ATC	5280
30	Val	Leu	Gly	His	Val	Ile	Leu	Ile	Cys	Gln	Leu	Pro	Leu	Ile	Ile	Ile	
					1745		1750				1755					1760	
	CCA	AAA	ATA	GAT	AAA	TTC	CAC	TCG	ATT	ATG	CTA	TTC	TGG	CTA	AAG	CCC	5328
	Pro	Lys	Ile	Asp	Lys	Phe	His	Ser	Ile	Met	Leu	Phe	Trp	Leu	Lys	Pro	
					1765				1770						1775		
	TCT	CGT	CAA	ATT	CGT	CCC	CCA	ATT	TAC	TCT	CTG	AAG	CAA	ACT	CGT	TTG	5376
35	Ser	Arg	Gln	Ile	Arg	Pro	Pro	Ile	Tyr	Ser	Leu	Lys	Gln	Thr	Arg	Leu	
				1780				1785						1790			
	CGT	AAG	CGT	ATG	GTC	AAG	AAG	TAC	TGC	TCT	TTG	TAC	TTT	TTA	GTA	TTG	5424
	Arg	Lys	Arg	Met	Val	Lys	Lys	Tyr	Cys	Ser	Leu	Tyr	Phe	Leu	Val	Leu	
				1795			1800						1805				
40	GCT	ATT	TTT	GCA	GGA	TGC	ATT	ATT	GGT	CCT	GCT	GTA	GCC	TCT	GCT	AAG	5472
	Ala	Ile	Phe	Ala	Gly	Cys	Ile	Ile	Gly	Pro	Ala	Val	Ala	Ser	Ala	Lys	
				1810			1815					1820					
	ATC	CAC	AAA	CAC	ATT	GGA	GAT	TCA	TTG	GAT	GGC	GTT	GTT	CAC	AAT	CTA	5520
45	Ile	His	Lys	His	Ile	Gly	Asp	Ser	Leu	Asp	Gly	Val	Val	His	Asn	Leu	
					1825		1830				1835					1840	
	TTC	CAA	CCA	ATA	AAT	ACA	ACC	AAT	AAT	GAC	ACT	GGT	TCC	CAA	ATG	TCA	5568
	Phe	Gln	Pro	Ile	Asn	Thr	Thr	Asn	Asn	Asp	Thr	Gly	Ser	Gln	Met	Ser	
					1845				1850						1855		
50	ACT	TAT	CAA	AGT	CAC	TAC	TAT	ACT	CAT	ACG	CCA	TCA	TTA	AAG	ACC	TGG	5616
	Thr	Tyr	Gln	Ser	His	Tyr	Tyr	Thr	His	Thr	Pro	Ser	Leu	Lys	Thr	Trp	

55

1860 1865 1870 5631

TCA ACT ATA AAA TAA
Ser Thr Ile Lys
1875

5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1876 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

15 Met Asn Thr Asp Gln Gln Pro Tyr Gln Gly Gln Thr Asp Tyr Thr Gln
1 5 10 15

Gly Pro Gly Asn Gly Gln Ser Gln Glu Gln Asp Tyr Asp Gln Tyr Gly
20 25 30

Gln Pro Leu Tyr Pro Ser Gln Ala Asp Gly Tyr Tyr Asp Pro Asn Val
35 40 45

Ala Ala Gly Thr Glu Ala Asp Met Tyr Gly Gln Gln Pro Pro Asn Glu
50 55 60

25 Ser Tyr Asp Gln Asp Tyr Thr Asn Gly Glu Tyr Tyr Gly Gln Pro Pro
65 70 75 80

Asn Met Ala Ala Gln Asp Gly Glu Asn Phe Ser Asp Phe Ser Ser Tyr
85 90 95

Gly Pro Pro Gly Thr Pro Gly Tyr Asp Ser Tyr Gly Gly Gln Tyr Thr
30 100 105 110

Ala Ser Gln Met Ser Tyr Gly Glu Pro Asn Ser Ser Gly Thr Ser Thr
115 120 125

Pro Ile Tyr Gly Asn Tyr Asp Pro Asn Ala Ile Ala Met Ala Leu Pro
35 130 135 140

Asn Glu Pro Tyr Pro Ala Trp Thr Ala Asp Ser Gln Ser Pro Val Ser
145 150 155 160

Ile Glu Gln Ile Glu Asp Ile Phe Ile Asp Leu Thr Asn Arg Leu Gly
165 170 175

40 Phe Gln Arg Asp Ser Met Arg Asn Met Phe Asp His Phe Met Val Leu
180 185 190

Leu Asp Ser Arg Ser Ser Arg Met Ser Pro Asp Gln Ala Leu Leu Ser
195 200 205

45 Leu His Ala Asp Tyr Ile Gly Gly Asp Thr Ala Asn Tyr Lys Lys Trp
210 215 220

Tyr Phe Ala Ala Gln Leu Asp Met Asp Asp Glu Ile Gly Phe Arg Asn
225 230 235 240

50 Met Ser Leu Gly Lys Leu Ser Arg Lys Ala Arg Lys Ala Lys Lys Lys
245 250 255

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	Asn	Lys	Lys	Ala	Met	Glu	Glu	Ala	Asn	Pro	Glu	Asp	Thr	Glu	Glu	Thr	
				260					265					270			
5	Leu	Asn	Lys	Ile	Glu	Gly	Asp	Asn	Ser	Leu	Glu	Ala	Ala	Asp	Phe	Arg	
			275					280					285				
	Trp	Lys	Ala	Lys	Met	Asn	Gln	Leu	Ser	Pro	Leu	Glu	Arg	Val	Arg	His	
			290				295					300					
10	Ile	Ala	Leu	Tyr	Leu	Leu	Cys	Trp	Gly	Glu	Ala	Asn	Gln	Val	Arg	Phe	
	305					310					315					320	
	Thr	Ala	Glu	Cys	Leu	Cys	Phe	Ile	Tyr	Lys	Cys	Ala	Leu	Asp	Tyr	Leu	
					325					330					335		
15	Asp	Ser	Pro	Leu	Cys	Gln	Gln	Arg	Gln	Glu	Pro	Met	Pro	Glu	Gly	Asp	
				340					345					350			
	Phe	Leu	Asn	Arg	Val	Ile	Thr	Pro	Ile	Tyr	His	Phe	Ile	Arg	Asn	Gln	
			355					360					365				
20	Val	Tyr	Glu	Ile	Val	Asp	Gly	Arg	Phe	Val	Lys	Arg	Glu	Arg	Asp	His	
	370						375					380					
	Asn	Lys	Ile	Val	Gly	Tyr	Asp	Asp	Leu	Asn	Gln	Leu	Phe	Trp	Tyr	Pro	
	385					390					395					400	
25	Glu	Gly	Ile	Ala	Lys	Ile	Val	Leu	Glu	Asp	Gly	Thr	Lys	Leu	Ile	Glu	
					405					410					415		
	Leu	Pro	Leu	Glu	Glu	Arg	Tyr	Leu	Arg	Leu	Gly	Asp	Val	Val	Trp	Asp	
				420					425					430			
30	Asp	Val	Phe	Phe	Lys	Thr	Tyr	Lys	Glu	Thr	Arg	Thr	Trp	Leu	His	Leu	
			435					440					445				
	Val	Thr	Asn	Phe	Asn	Arg	Ile	Trp	Val	Met	His	Ile	Ser	Ile	Phe	Trp	
		450					455					460					
35	Met	Tyr	Phe	Ala	Tyr	Asn	Ser	Pro	Thr	Phe	Tyr	Thr	His	Asn	Tyr	Gln	
	465					470					475					480	
	Gln	Leu	Val	Asp	Asn	Gln	Pro	Leu	Ala	Ala	Tyr	Lys	Trp	Ala	Ser	Cys	
					485					490					495		
40	Ala	Leu	Gly	Gly	Thr	Val	Ala	Ser	Leu	Ile	Gln	Ile	Val	Ala	Thr	Leu	
				500					505					510			
	Cys	Glu	Trp	Ser	Phe	Val	Pro	Arg	Lys	Trp	Ala	Gly	Ala	Gln	His	Leu	
			515					520					525				
45	Ser	Arg	Arg	Phe	Trp	Phe	Leu	Cys	Ile	Ile	Phe	Gly	Ile	Asn	Leu	Gly	
		530					535					540					
	Pro	Ile	Ile	Phe	Val	Phe	Ala	Tyr	Asp	Lys	Asp	Thr	Val	Tyr	Ser	Thr	
		545				550					555					560	
50	Ala	Ala	His	Val	Val	Ala	Ala	Val	Met	Phe	Phe	Val	Ala	Val	Ala	Thr	
					565					570					575		
	Ile	Ile	Phe	Phe	Ser	Ile	Met	Pro	Leu	Gly	Gly	Leu	Phe	Thr	Ser	Tyr	
				580					585					590			
55	Met	Lys	Lys	Ser	Thr	Arg	Arg	Tyr	Val	Ala	Ser	Gln	Thr	Phe	Thr	Ala	

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	595		600		605													
5	Ala	Phe	Ala	Pro	Leu	His	Gly	Leu	Asp	Arg	Trp	Met	Ser	Tyr	Leu	Val		
	610						615					620						
	Trp	Val	Thr	Val	Phe	Ala	Ala	Lys	Tyr	Ser	Glu	Ser	Tyr	Tyr	Phe	Leu		
	625					630					635					640		
	Val	Leu	Ser	Leu	Arg	Asp	Pro	Ile	Arg	Ile	Leu	Ser	Thr	Thr	Ala	Met		
10					645					650					655			
	Arg	Cys	Thr	Gly	Glu	Tyr	Trp	Trp	Gly	Ala	Val	Leu	Cys	Lys	Val	Gln		
				660					665					670				
	Pro	Lys	Ile	Val	Leu	Gly	Leu	Val	Ile	Ala	Thr	Asp	Phe	Ile	Leu	Phe		
15			675				680						685					
	Phe	Leu	Asp	Thr	Tyr	Leu	Trp	Tyr	Ile	Ile	Val	Asn	Thr	Ile	Phe	Ser		
	690						695					700						
	Val	Gly	Lys	Ser	Phe	Tyr	Leu	Gly	Ile	Ser	Ile	Leu	Thr	Pro	Trp	Arg		
20	705					710					715					720		
	Asn	Ile	Phe	Thr	Arg	Leu	Pro	Lys	Arg	Ile	Tyr	Ser	Lys	Ile	Leu	Ala		
					725					730					735			
	Thr	Thr	Asp	Met	Glu	Ile	Lys	Tyr	Lys	Pro	Lys	Val	Leu	Ile	Ser	Gln		
25				740					745					750				
	Val	Trp	Asn	Ala	Ile	Ile	Ile	Ser	Met	Tyr	Arg	Glu	His	Leu	Leu	Ala		
			755					760					765					
	Ile	Asp	His	Val	Gln	Lys	Leu	Leu	Tyr	His	Gln	Val	Pro	Ser	Glu	Ile		
30			770			775						780						
	Glu	Gly	Lys	Arg	Thr	Leu	Arg	Ala	Pro	Thr	Phe	Phe	Val	Ser	Gln	Asp		
	785					790					795					800		
	Asp	Asn	Asn	Phe	Glu	Thr	Glu	Phe	Phe	Pro	Arg	Asp	Ser	Glu	Ala	Glu		
				805						810					815			
35	Arg	Arg	Ile	Ser	Phe	Phe	Ala	Gln	Ser	Leu	Ser	Thr	Pro	Ile	Pro	Glu		
				820					825					830				
	Pro	Leu	Pro	Val	Asp	Asn	Met	Pro	Thr	Phe	Thr	Val	Leu	Thr	Pro	His		
			835				840						845					
40	Tyr	Ala	Glu	Arg	Ile	Leu	Leu	Ser	Leu	Arg	Glu	Ile	Ile	Arg	Glu	Asp		
	850					855						860						
	Asp	Gln	Phe	Ser	Arg	Val	Thr	Leu	Leu	Glu	Tyr	Leu	Lys	Gln	Leu	His		
	865					870					875				880			
45	Pro	Val	Glu	Trp	Glu	Cys	Phe	Val	Lys	Asp	Thr	Lys	Ile	Leu	Ala	Glu		
				885						890					895			
	Glu	Thr	Ala	Ala	Tyr	Glu	Gly	Asn	Glu	Asn	Glu	Ala	Glu	Lys	Glu	Asp		
				900				905						910				
50	Ala	Leu	Lys	Ser	Gln	Ile	Asp	Asp	Leu	Pro	Phe	Tyr	Cys	Ile	Gly	Phe		
			915					920					925					
	Lys	Ser	Ala	Ala	Pro	Glu	Tyr	Thr	Leu	Arg	Thr	Arg	Ile	Trp	Ala	Ser		
55							935					940						

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	Leu Arg Ser Gln Thr	Leu Tyr Arg Thr Ile	Ser Gly Phe Met Asn Tyr
	945	950	955 960
5	Ser Arg Ala Ile Lys	Leu Leu Tyr Arg Val	Glu Asn Pro Glu Ile Val
	965	970	975
	Gln Met Phe Gly Gly	Asn Ala Glu Gly	Leu Glu Arg Glu Leu Glu Lys
	980	985	990
10	Met Ala Arg Arg Lys Phe	Lys Phe Leu Val Ser Met	Gln Arg Leu Ala
	995	1000	1005
	Lys Phe Lys Pro His Glu	Leu Glu Asn Ala Glu	Phe Leu Leu Arg Ala
	1010	1015	1020
15	Tyr Pro Asp Leu Gln Ile	Ala Tyr Leu Asp Glu Glu	Pro Pro Leu Thr
	1025	1030	1035 1040
	Glu Gly Glu Glu Pro Arg	Ile Tyr Ser Ala Leu Ile	Asp Gly His Cys
	1045	1050	1055
20	Glu Ile Leu Asp Asn Gly	Arg Arg Arg Pro Lys Phe	Arg Val Gln Leu
	1060	1065	1070
	Ser Gly Asn Pro Ile Leu	Gly Asp Gly Lys Ser Asp	Asn Gln Asn His
	1075	1080	1085
25	Ala Leu Ile Phe Tyr Arg	Gly Glu Tyr Ile Gln Leu	Ile Asp Ala Asn
	1090	1095	1100
	Gln Asp Asn Tyr Leu Glu	Glu Cys Leu Lys Ile Arg	Ser Val Leu Ala
	1105	1110	1115 1120
30	Glu Phe Glu Glu Leu Asn	Val Glu Gln Val Asn Pro	Tyr Ala Pro Gly
	1125	1130	1135
	Leu Arg Tyr Glu Glu Gln	Thr Thr Asn His Pro Val	Ala Ile Val Gly
	1140	1145	1150
35	Ala Arg Glu Tyr Ile Phe	Ser Glu Asn Ser Gly Val	Leu Gly Asp Val
	1155	1160	1165
	Ala Ala Gly Lys Glu Gln	Thr Phe Gly Thr Leu Phe	Ala Arg Thr Leu
	1170	1175	1180
40	Ser Gln Ile Gly Gly Lys	Leu His Tyr Gly His Pro	Asp Phe Ile Asn
	1185	1190	1195 1200
	Ala Thr Phe Met Thr Thr	Arg Gly Gly Val Ser Lys	Ala Gln Lys Gly
	1205	1210	1215
45	Leu His Leu Asn Glu Asp	Ile Tyr Ala Gly Met Asn	Ala Met Leu Arg
	1220	1225	1230
	Gly Gly Arg Ile Lys His	Cys Glu Tyr Tyr Gln Cys	Gly Lys Gly Arg
	1235	1240	1245
50	Asp Leu Gly Phe Gly Thr	Ile Leu Asn Phe Thr Thr	Lys Ile Gly Ala
	1250	1255	1260
	Gly Met Gly Glu Gln Met	Leu Ser Arg Glu Tyr Tyr	Tyr Leu Gly Thr
	1265	1270	1275 1280
55	Gln Leu Pro Val Asp Arg	Phe Leu Thr Phe Tyr Tyr	Ala His Pro Gly
	1285	1290	1295

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5 Phe His Leu Asn Asn Leu Phe Ile Gln Leu Ser Leu Gln Met Phe Met
 1300 1305 1310
 Leu Thr Leu Val Asn Leu Ser Ser Leu Ala His Glu Ser Ile Met Cys
 1315 1320 1325
 10 Ile Tyr Asp Arg Asn Lys Pro Lys Thr Asp Val Leu Val Pro Ile Gly
 1330 1335 1340
 Cys Tyr Asn Phe Gln Pro Ala Val Asp Trp Val Arg Arg Tyr Thr Leu
 1345 1350 1355 1360
 Ser Ile Phe Ile Val Phe Trp Ile Ala Phe Val Pro Ile Val Val Gln
 1365 1370 1375
 15 Glu Leu Ile Glu Arg Gly Leu Trp Lys Ala Thr Gln Arg Phe Phe Cys
 1380 1385 1390
 His Leu Leu Ser Leu Ser Pro Met Phe Glu Val Phe Ala Gly Gln Ile
 1395 1400 1405
 20 Tyr Ser Ser Ala Leu Leu Ser Asp Leu Ala Ile Gly Gly Ala Arg Tyr
 1410 1415 1420
 Ile Ser Thr Gly Arg Gly Phe Ala Thr Ser Arg Ile Pro Phe Ser Ile
 1425 1430 1435 1440
 25 Leu Tyr Ser Arg Phe Ala Gly Ser Ala Ile Tyr Met Gly Ala Arg Ser
 1445 1450 1455
 Met Leu Met Leu Leu Phe Gly Thr Val Ala His Trp Gln Ala Pro Leu
 1460 1465 1470
 30 Leu Trp Phe Trp Ala Ser Leu Ser Ser Leu Ile Phe Ala Pro Phe Val
 1475 1480 1485
 Phe Asn Pro His Gln Phe Ala Trp Glu Asp Phe Phe Leu Asp Tyr Arg
 1490 1495 1500
 35 Asp Tyr Ile Arg Trp Leu Ser Arg Gly Asn Asn Gln Tyr His Arg Asn
 1505 1510 1515 1520
 Ser Trp Ile Gly Tyr Val Arg Met Ser Arg Ala Arg Ile Thr Gly' Phe
 1525 1530 1535
 40 Lys Arg Lys Leu Val Gly Asp Glu Ser Glu Lys Ala Ala Gly Asp Ala
 1540 1545 1550
 Ser Arg Ala His Arg Thr Asn Leu Ile Met Ala Glu Ile Ile Pro Cys
 1555 1560 1565
 45 Ala Ile Tyr Ala Ala Gly Cys Phe Ile Ala Phe Thr Phe Ile Asn Ala
 1570 1575 1580
 Gln Thr Gly Val Lys Thr Thr Asp Asp Asp Arg Val Asn Ser Val Leu
 1585 1590 1595 1600
 50 Arg Ile Ile Ile Cys Thr Leu Ala Pro Ile Ala Val Asn Leu Gly Val
 1605 1610 1615
 Leu Phe Phe Cys Met Gly Met Ser Cys Cys Ser Gly Pro Leu Phe Gly
 1620 1625 1630
 55 Met Cys Cys Lys Lys Thr Gly Ser Val Met Ala Gly Ile Ala His Gly

	1635	1640	1645
5	Val Ala Val Ile Val His 1650	Ile Ala Phe Phe Ile 1655	Val Met Trp Val Leu 1660
	Glu Ser Phe Asn Phe Val Arg Met Leu Ile 1665	Gly Val Val Thr Cys Ile 1670	1675 1680
10	Gln Cys Gln Arg Leu Ile Phe His Cys Met Thr Ala Leu Met Leu Thr 1685	1690	1695
	Arg Glu Phe Lys Asn Asp His Ala Asn Thr Ala Phe Trp Thr Gly Lys 1700	1705	1710
15	Trp Tyr Gly Lys Gly Met Gly Tyr Met Ala Trp Thr Gln Pro Ser Arg 1715	1720	1725
	Glu Leu Thr Ala Lys Val Ile Glu Leu Ser Glu Phe Ala Ala Asp Phe 1730	1735	1740
20	Val Leu Gly His Val Ile Leu Ile Cys Gln Leu Pro Leu Ile Ile Ile 1745	1750	1755 1760
	Pro Lys Ile Asp Lys Phe His Ser Ile Met Leu Phe Trp Leu Lys Pro 1765	1770	1775
25	Ser Arg Gln Ile Arg Pro Pro Ile Tyr Ser Leu Lys Gln Thr Arg Leu 1780	1785	1790
	Arg Lys Arg Met Val Lys Lys Tyr Cys Ser Leu Tyr Phe Leu Val Leu 1795	1800	1805
30	Ala Ile Phe Ala Gly Cys Ile Ile Gly Pro Ala Val Ala Ser Ala Lys 1810	1815	1820
	Ile His Lys His Ile Gly Asp Ser Leu Asp Gly Val Val His Asn Leu 1825	1830	1835 1840
35	Phe Gln Pro Ile Asn Thr Thr Asn Asn Asp Thr Gly Ser Gln Met Ser 1845	1850	1855
	Thr Tyr Gln Ser His Tyr Tyr Thr His Thr Pro Ser Leu Lys Thr Trp 1860	1865	1870
40	Ser Thr Ile Lys 1875		

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Claims

1. A substantially pure ECB binding peptide comprising at least 46 contiguous amino acid residues of SEQ ID NO:2.
2. A substantially pure ECB binding peptide, as in Claim 1 comprising the amino acid sequence defined by residues 605 to 650 of SEQ ID NO:2.
3. An isolated nucleic acid compound encoding a peptide of Claim 1 or Claim 2.
4. An isolated nucleic acid encoding a peptide of Claim 1 wherein said nucleic acid has a sequence selected from the group consisting of:

- (a) (a) residues 1747 to 2016 of SEQ ID NO:1; or
(b) a nucleic acid compound complementary to (a).

5. A vector comprising an isolated nucleic acid compound of Claim 3.

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6. A host cell containing a vector of Claim 5.

7. A method for constructing a recombinant host cell having the potential to express an ECB binding domain of SEQ ID NO:2, said method comprising introducing into said host cell by any suitable means a vector of Claim 5.

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8. A method for expressing an ECB binding domain of SEQ ID NO:2 in the recombinant host cell of Claim 7, said method comprising culturing said recombinant host cell under conditions suitable for gene expression.

9. A method for identifying compounds that bind an ECB binding domain, comprising the steps of:

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a) admixing in a suitable reaction buffer

- i) a substantially pure ECB binding peptide, as claimed in Claim 1; and
ii) a test inhibitory compound;

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b) measuring by any suitable means a binding between said peptide and said compound.

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(12) **EUROPEAN PATENT APPLICATION**

(88) Date of publication A3:
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(54) **Echinocandin binding domain of 1,3-Beta-glucan synthase**

(57) The invention relates to a substantially purified ECB binding domain of 1,3- β -glucan synthase, comprising an at least 46 amino acid peptide fragment or fusion protein of glucan synthase that binds echinocandins,

useful in a method for identifying new antifungal compounds. Also disclosed are nucleic acid molecules that encode said peptide.



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EUROPEAN SEARCH REPORT

Application Number
EP 98 31 0497

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	WO 95 10625 A (DOUGLAS CAMERON M ;KAHN JENNIFER NIELSEN (US); PARENT STEPHEN ARTH) 20 April 1995 (1995-04-20)	1-8	C12N15/54 C12N9/10 C12N1/15 C12N1/21 C12Q1/48
Y	* page 13, line 30 - line 34; claim 9; figures 6,7; example 31 *	9	
Y	DOUGLAS C M ET AL : "Identification of the FKS1 gene of Candida albicans as the essential target of 1,3-beta-D-glucan synthase inhibitors" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 41, no. 11, November 1997 (1997-11), pages 2471-2479, XP000107858 United States * abstract *	9	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C12N C12Q
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 2 May 2002	Examiner Meacock, S
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons § : member of the same patent family, corresponding document</p>			

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82